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SCREENING OF THE ADVERSE EFFECTS OF SUBSTANCES OF ABUSE ON KIDNEY FUNCTIONS: AN EGYPTIAN SINGLE CENTER STUDY

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ABSTRACT

Recently, substance abuse has become an increasingly prevalent problem all over the world. The kidneys - As an excretory organs- are highly vulnerable to the toxic effect of abuse substances. This study was aiming to study the nephrotoxic effect of commonly abused substances in Egypt on the kidney functions among abusers. In this study we have investigated the effects of commonly abused drugs in Egypt (tramadol, cannabis, opiates, barbiturates and benzodiazepines)on kidney functions (serum creatinine and blood urea nitrogen [BUN]).One Hundred - eighty four persons were screened for these substances using enzyme multiplied immunoassay technique (EMIT), positive samples were further confirmed by gas chromatography-mass spectroscopy (GC- MS). Eighty-nine cases showed positive results by GC-MS. Tramadol was the most commonly abused substance in the studied population (29 cases). Our data showed that substance abuse significantly increased level of serum creatinine and BUN among abusers (The mean level of serum creatinine level was statistically higher in abusers (1.08±0.4mg/dl) compared with non- abusers (0.85±0.25 mg/dl), this difference was statistically significant (*P*=0.0002).Thirty-seven abusers showed serum creatnine level ≥1.2 mg/dl. The mean level of BUN was also statistically higher in abusers (13.45±3mg/dl) compared with non- abusers (11.7±2.5 mg/dl) compared with non- abusers (11.7±2.5 mg/dl), again the difference was statistically significant (*p*=0.0007).Tramadol and other opioids were shown to be the most toxic to the kidneys. Kidney injury marker 1 (KIM1) and albumin/creatinine ratio (ACR) showed that tramadol renal injury is mainly tubular, while other opiates produce tubular and glomerular adverse effect. Substance abuse- especially tramadol and other opioids - significantly impairs the kidney function in healthy abusers, so they should be considered as a risk factor for renal injury.

Keywords: Tramadol, Substance abuse, KIM1, Albumin/createnine ratio

INTRODUCTION

Substance abuse is a widely prevalent worldwide problem with many social, behavioral and medical negative drawbacks. A lot of psychoactive substances are identified indifferent populations with different governmental and non-governmental plans to control their handling and limit their wide spread use [1]. Substances abuses are commonly

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referred to as psychoactive substances. They are classified according to their central effects into anxiolytics (as benzodiazepine), stimulants (as cocaine and nicotine), euphoriants (as Ecstasy), depressants as

Tramadol is a commonly abused drug in Egypt [1). It is a synthetic opioid. In addition to its week opioid receptor antagonism, It action is exerted via inhibition of norepinephrine and serotonin re-uptake [2-4]. Tramadol abuse is mainly due to its popularity among abusers and over prescription as an analgesic. Furthermore, tramadol is promoted in many online drug stores and media as a remedy for ejaculatory disorders and to increase sexual pleasure as promoted. Other opioids as morphine, heroin and codeine are commonly abused in Egyptas well [5].

Cannabis is a commonly abused euphoriant in Egypt with widely accepted believes among abusers as it is a harmless natural product. Furthermore, they believe that it improves sexual potency and increases orgasm [6].

Central nervous depressants are widely known among addicts in Egypt. Benzodaizepams (diazepam (Valium) and alprazolam (Xanax)) are the most commonly abused central depressants in Egypt while barbiturates as phenobarbital (Luminal Sodium) are less widely used as psychoactive drugs.

The kidneys are highly susceptible to toxic injuries due to different causes [7]. Firstly, They receive up to one quarter of cardiac output and so they are exposed to large proportions of the circulating xentbiotics. Secondly, kidneys are metabolically active organs and thus they are vulnerable to active toxic metabolites of some nontoxic parent xenobiotics [8]. Thirdly, Kidneys paranychyma are exposed to higher xenobiotics concentration after water reabsorption in the renal tubules with more concentration of the urine solutes. Furthermore, renal tissues are susceptible to attack by the immune system [9].

The aim of the current work was to evaluate kidney functions among addict, abusing commonly used psychoactive drugs in Egypt (tramadol, cannabis, opiates, barbiturates and benzodiazepines) using serum creatinine and blood urea nitrogen (BUN) as the most frequently used serologic indicators for the diagnosis of renal dysfunction. To investigate for the possible site of psychoactive substances induced renal damage, kidney injury marker 1 (KIM1) and albumin/creatinine ratio (ACR) were used as markers for renal tubular and glomerular injuries, respectively [10,11].

Patients and methods

The study was conducted in Mansoura University Hospitals, Dakahlyia, Egypt between November 2012 and November 2013. The facility is a tertiary health care center draining wide area to the east of the Egyptian Deltaregion.

The protocol of the present work was approvedby Mansoura Faculty of Medicine research ethical committee. Confidentiality was respected. Signed informed consents were taken from all participants. Two hundred and twenty persons were enrolled in the study. Participants were patients' relatives or friends attending to the university hospital. They were not seeking medical advice they came just accompanying their sick relatives or friends. For all participants, complete history was taken including: age, residency, educational level, occupation, history of any chronic diseases and any medications. Persons with history of chronic kidney diseases, diabetes, and hypertension or on steroids therapy or any other concurrent medical prescriptions were excluded from the study. Persons with positive screening for hepatitis B and C viruses and with AIDS were excluded as these virsuses may cause adverse effect on the kidney functions. No stipend was provided; however each participant was informed about the results of their livers and kidneys functions laboratory tests. Renal ultrasonography was performed to exclude any renal parenchymal gross lesions. Urine test:

For drug abuse screening, 20 ml urine was obtained from each participant and in a dry, labeled container. Samples were screened for 5 substances abuse (opiate, cannabis, benzodiazepines, barbiturates and tramadol) by enzyme multiplied immunoassay technique (EMIT) using Emit® d.a.u. TM (drug of abuse in urine). After the initial screens, positive cases were confirmed by gas chromatography-mass spectroscopy (GC-MS). Other abuse drugs were not screened in our study as cocaine and amphetamine as they are not available and not used in our locality.

Markers for renal tubular damage [kidney injury molecule-1 (KIM-1)] and glomerular damage [urine albumin creatinine ratios (ACR)] were studied in urine samples of tramadol and opiates abusers.

For KIM-1 assay, ELISA plate (MaxiSorp; Nunc, Naperville, IL, USA) were used. Briefly, 100µL of urine sample were incubated /wells for three hours at room temperature. Wells were washed for four times with PBST, Then biotinylated AKG7 antibody and HRP-conjugated streptavidin were added. The urinary KIM-1 was estimated in ng/ml.

For microalbuminuria, an early morning samples were used. Urine samples of tramadol, opiate abusers and controls were assayed for albumin and creatinine concentrations. Albumin concentration was measured by Micrototal Protein (MT-P) (Spectrum, Egyptian company for Biotechnology (S.A.E), Cairo, Egypt), following the manufacturer protocol. Urine creatinine concentrations were performed by Buffered Kinetic jaffé reaction without deproteinization using creatinine Jaffe kit (Spectrum, Egyptian company for Biotechnology (S.A.E), Cairo, Egypt).

Blood test:

Blood tramadol levels were estimated by gas chromatography-mass spectroscopy (GC-MS) (Hewlett Packard, 6890 series), following Goeringer et al. (1997) [12].

Kidney functions (Serum creatinine and blood urea nitrogen (BUN)) were done for all participants.

RESULTS

Demographic data of the studied cases (table 1)

Among 220 interviewed and examined persons, only 184 (158 males 26 females) satisfied the inclusion criteria of the study. Thirty-six persons were excluded. i.e 18 refused to be screen for psychoactive drugs after initial consent, 7 were hypertensives, 6 were diabetics , 4 were asthmatics on corticosteroid therapy while one case was excluded as renal ultra-sonography showed back pressure and increased parenchymal echogenicity due to left ureteric stone. Age of persons enrolled in the study ranged between 19-46years (with a mean age of 26.4±8). Majority of participants 42 persons [22.7%] were manual workers, while 29 participants (15.6%) have reported no occupations.

Sixty –Eight (36.6%) participants were living in rural area, while the remaining participants came from urban area.

Prevalence of substance abuse

One Hundred and Twenty eight case showed positive urine samples by EMIT. Positive cases were further confirmed by GC-MS. GC-MS results confirmed only 89 positive cases: 18 cases have showed positive results for more than one psychoactive substance (mostly tramadol with cannabis [9 persons]). According to GC-MS data considering single substance abuse, 29 cases were positive for tramadol, 21 cases were positive for cannabinoids, 16 cases were positive for opiates, 8 cases were positive for benzodiazepine and 6 cases were positive for barbiturates. (EMIT screen and GC-MS results are shown in figure (1).

Kidney functions in the studied cases

The mean level of serum creatinine level was statistically higher in abusers (1.08±0.4mg/dl) compared with non- abusers (0.85±0.25 mg/dl), this difference was statistically significant. (*P*=0.0002;Mann-Whitney).

Thirty-seven abusers showed serum creatnine level ≥1.2 mg/dl. The mean level of BUN was also statistically higher in abusers (13.45±3mg/dl) compared with non-abusers (11.7±2.5 mg/dl), again the difference was statistically significant (*P*=0.0007; Mann-Whitney).Sixty-two persons showed BUN levels above non abusers mean value.

Cases with increased creatinine above normal had showed BUN/creatinine ratio blow than (Data are shown in figure 2).

For tramadol and opiate abusers with significant changes in the renal function test (serum creatinine and blood urea nitrogen) , KIM1 and ACR were done and studied in comparison to the controls non abusers group. KIM1 showed significant increase in both tramadol and opiates abusers group (0.28±0.25 ng/ml and 0.39±0.34ng/ml respectively) in comparison to controls (0.047±0.0043 ng/ml) (P-value=0.0002; one way ANOVA). Dunnet's multiple comparisons post-test showed that increased KIM1 values were more significant among other opiates abusers group in comparisons to controls (Figure 3).

ACR showed only significant increase in opiates abusers in comparison to controls (P- value=0.0015; Fisher exact test) (Table 2).

Table 1. Demographic characters of the studied population

Parameter		Non abusers (95)	Abusers (89)	P-value
Age		32.5±5.3	22.5± 3.9	0.002** (a)
Gender	Male	76	82	0.02* (b)
	Female	19	7	
Work	Manual workers	12	30	0.007** (c)
	Governmental	28	18	
	Farmers	20	16	
	University	21	10	
	Not working	14	15	
Residency	Urban	57	55	<0.541 (b)
	Rural	38	30	
Education	Universities	45	23	0.0112* (c)
	High school	29	37	
	Non-educated	21	29	

(a) means p-value estimated by chi square (b) means p-value estimated by Fisher exact and (c) means p-value estimated by Mann-Whitney test.

Table 2. Difference in albumin/creatinine ratios among controls (nonabusers) and tramadol and other opiates abusers. High ACR means ACR ≥3.5 mg/mmol (female) or ≥2.5 mg/mmol (male)

	Normal ACR	High ACR	Total	
Non abusers	84 (88.55)	11 (11.5%)	95 (100%)	0.075
Tramadol abusers	22 (72.5%)	8 (27.5%)	29 (100%)	
Non abusers	84 (88.55)	11 (11.5%)	95 (100%)	0.0015**
Opiates abusers	9 (56.25%)	7 (43.75%)	16 (100%)	

Fig 1. Screening of substance of abuse [tramadol (TRA), cannabis (CAN), Opiates (OP), barbiturates (BAR) and benzodiazepines (BZ)] in the studied group by EMIT and TLC with estimation of EMIT specificity. EMIT screen data was confirmed by TLC.

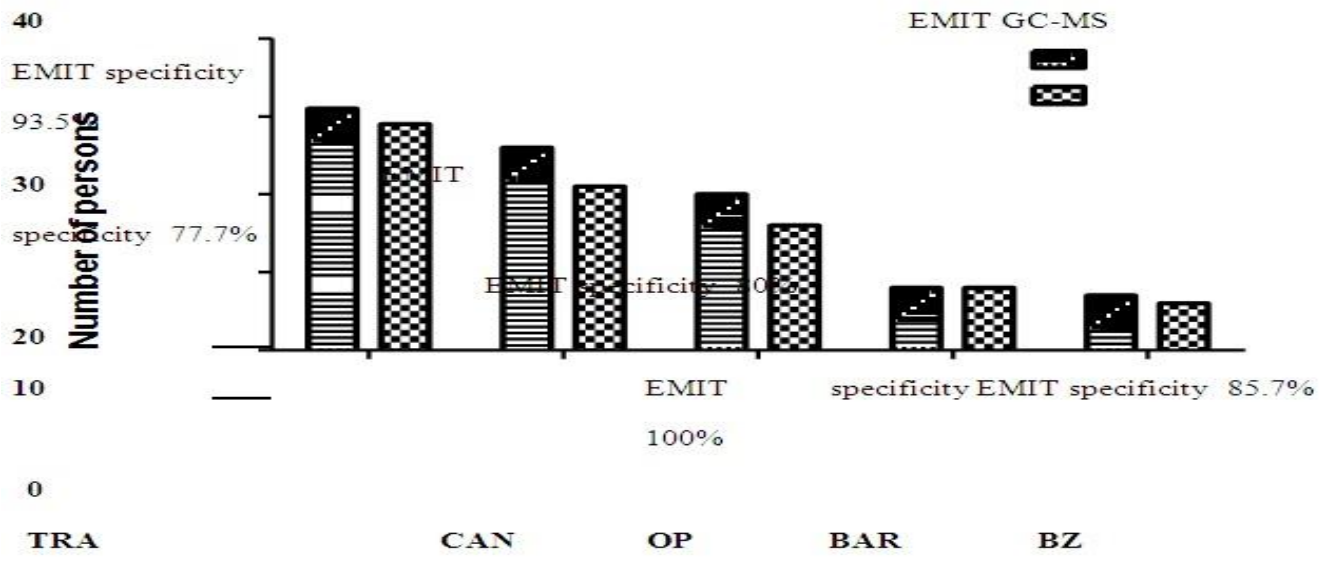


Fig 2. Effect of substances of abuse [tramadol (TRA), cannabis (CAN), Opiates (OP), barbiturates (BARB) and benzodiazepines (BZ)] on serum createnine and blood urea nitrogen (BUN). Data are shown as means and standard deviation. (* indicate p-value <0.05) when compared with vehicle control treated group).

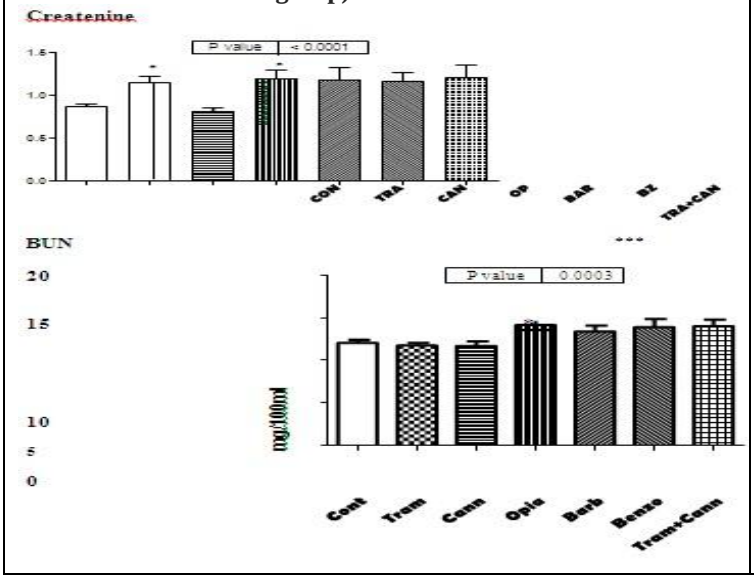
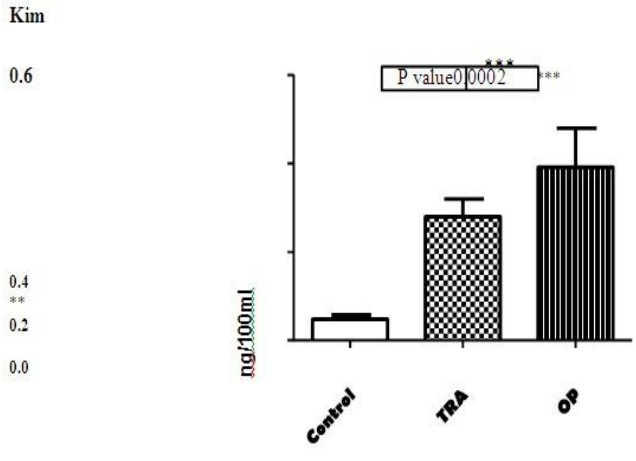


Fig 3. Difference in kidney injury marker 1 (KIM1) among controls (nonabusers) and tramadol (TRA) and other opiates (OP) abusers. Data are shown as means and standard deviation. (indicate p-value <0.01, while *** indicated p-value<0.001 when compared with vehicle control treated group).**



DISCUSSION

Our data revealed that the 48.4% of the studied population had positive samples for abuse substances by GC-MS. There was no significant difference between abusers and non abusers regarding to their ages. This finding is not in accordance with results of previous studies, who reported a higher prevalence of substance abuse among young people. This may be due to changes in abuse prevalence among the different age groups, but we could not conclude that as the majority of the studied cases ages were below 30 years old (67% of the studied populations).

There was a statistically significant difference between both the abusers and non-abusers regarding their gender distribution. Fifty-four % of studied males were found positive to the screened psychoactive drugs, while only 27% of enrolled females showed positive GC-MS results [13,14]. This male higher prevalence of substance abuse is in agreement [15,16].

Interestingly, our study showed higher prevalence of abuse among manual workers (71% of studied manual workers were abusers). This is may be due to their high incomes in addition to false concepts that these substances will increase performance and reduce their fatigue and will give them better life quality. In our studied participants, we did not find significant difference in abuse prevalence in relation to residency. Furthermore the study showed more prevalence of abuse among non-educated and high school graduates in comparison to the universities graduates and students.

We have confirmed EMIT data regarding abuse with GC-MS to overcome data fallacies due to false positive and false negatives EMIT results. However, screening with EMIT will reduce the cost of the specific and sensitive GC-MS as it will reduce the number of samples because only positive EMIT samples will proceed to GC-MS analysis for more data robustness [17].

The present work showed that tramadol was the commonest abused drugs among abusers. The previous studies, which is not consistent with the result of previous studies done in Egypt which showed cannabis to be the commonest (***6***). This may be due to change patterns of abuse as tramadol is widely used as analgesic. Also its low price and wider availability may give it more popularity among abusers.

Renal dysfunction can be induced by either injury to the glomeruli or the tubules leading to a decrease in the normal glomerular filtration rates (GFR). This will be in parallel with increased serum levels of the blood markers as blood urea nitrogen and creatinine. However, the relationship between these markers and the level of GFR is not linear, as a small elevation in serum levels of these biomarkers always detected with marked decrease in renal function [18].

In the present work, tramadol was found to significantly increase serum creatinine and BUN among its abusers in comparison to controls with BUN/creatinine ratio <10/1 which means, mostly, intrarenal cause of the elevated levels of the renal biomarkers [19]. This increase may be explained by frequently reported seizures among tramadol abusers. Seizures are common side effect of tramadol even within the therapeutic doses. These repeated seizures can precipitate rhabdomylosis and myoglinurea with affection of kidney functions [20]. Moreover, Muller and Wilsmann [21] showed that tramadol use is associated with sympathetic stimulation due to increased norepinephrine release within its therapeutic concentrations. So it is expected that tramadol may decrease the renal blood flow, hence affecting glomerular filtration and renal functions especially with higher serum levels reported among abusers. We also suggest this increase in creatinine and BUN among abusers may be caused by negative protein balance as abused opioids as heroin are known to exert a direct myotoxic effect [22], So addict will spend all their money on tramadol with negligence of proper healthy balanced diet. Our findings are not against the recommendations to use tramadol to control pain among patients with renal impairment with dose adjustment [23] as our study was dealing with abusers with expected very high serum levels, even these abusers may tolerate toxic serum levels.

Regarding cannabis, it was found that cannabis did not significantly affect levels of serum creatinine and BUN. Interestingly, our data showed that cannabis in combination with tramadol decreases its harmful effect. That is may be due to cannabis action as antioxidant shown in previous studies as [24] in neuronal cell cultures. Our results are in accordance with what reported previously in literature, which did not show that cannabis affects the kidney function except in a single case study which reported right kidney infarction in a patient with heavy cannabis abuse after exclusion of primary or secondary hypercoagulable state or an underlying neoplasm [25].

Regarding opiates abusers, we have found that opiates negatively affected kidney functions. Opiates, especially heroin, were reported to affect the kidney via several ways. Opiate coma may induce pressure necrosis and rhabdomyolysis with myoglobinurea, associated hypotension, hypoxia, acidosis and dehydration may aggravate the condition [26]. Also glomerulonephritis may complicate addiction-related infections [27]. Additionally heroin associated nephropathy was first described in 1970s and 1980s, presenting as nephrotic syndrome and progressing rapidly to end-stage renal failure [28].

Regarding barbiturates, we did not find significant effect on serum creatinine or BUN. That is in accordance with previous barbiturates literature as there was no evidence that the conventional barbiturates can cause kidney injuries [29, 30].

Benzodiazepine abusers in the present work did not shown significant changes in comparison to the control non abuser groups regarding their kidney functions. Acute renal failure had been reported following inadvertent intraarterial

temazepam injection which causes rhabdomyolysis and myoglobinurea due to limb ischemia and muscle necrosis [31]. We did not find this effect in our study as temazepam is now only available as oral form.

The present study data showed that tramadol induced renal damage is mainly tubular while other opiates can induced both tubular and glomerular renal damages. This is in accordance [32] who had reported histopathological renal injuries in the forms of focal cortico-medullary mineralization with focal regeneration in tubular epithelium in association with intratubular crystal deposition after long-term use of LAAM(levo-α- acetylmethadol). Moreover [33] reported that morphine intake caused renal tubular vacuolization, with mononuclear cell infiltration and renal foci of necrosis and haemorrhage in rats receiving morphine, while minimal histopathological changes, limited only to tubular cells, were found in kidneys of rats treated with tramadol.

Taking into considerations that when blood urea nitrogen (BUN) or serum creatinine exceeds the upper limit of normal, GFR is already reduced by more than 50% [18] our data has a very high clinical significance. These findings should raise the attention of nephrologists to consider tramadol or other opiates abuse as a cause for abnormal levels in serum creatinine and BUN among otherwise healthy middle ages persons. Also substance abuse should be considered a risk factor for more renal impairment in concomitance with any other renal problems. In addition, substance abuse should be considered as a bad prognostic factor in any renal disorders. Also, the high prevalence of tramadol and other opioid drugs abuse in Egypt is indicating newer planning to control handling of opioid drugs in Egypt with more limitations on their medical prescriptions.

Demographic characters of participants involved in the study.

CONCLUSION

Results of our study allow us to conclude that substance abuse caused higher levels of serum creatinine and BUN among abusers especially who were dependant on tramadol and other opioids. These findings still need further confirmation regarding their robustness with larger studies enrolling large number of population. In addition, mechanisms of the effect of psychoactive substances on kidneys are in need for further mechanics studies. However, we can conclude that substance abuse can worsen any pathological renal condition.

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None

CONFLICT OF INTEREST

Authors declared no conflict of interest

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